Microcytic Anemia

Introduction

This lesson is about distinguishing the microcytic anemias from one another. Sideroblastic anemia is also microcytic, but was covered in Anemia #2: *Disorders of Heme Synthesis*. This lesson tackles the remaining microcytic anemias. There are two broad classes—iron and globin diseases. Those that are disorders of **iron**, and therefore affect heme formation, are acquired disorders, and are **iron deficiency anemia** and **anemia of chronic inflammatory disease**. Those that are disorders of globin are inherited genetic diseases of globin synthesis, and are **α-thalassemia** and **β-thalassemia**.

To arrive at this lesson following the approach to anemia, your patient would have been anemic, the MCV < 80 (i.e., microcytic), and the reticulocyte count low, indicating a production anemia. All microcytic anemias are production anemias. The next test to order in the setting of microcytic anemia is the anemia labs, the **total iron-binding capacity** (TIBC, which is the same thing as saying "transferrin"), **serum iron**, the stored form of iron (**ferritin**), and the relative saturation of transferrin by serum iron (**% saturation**).

It should come as no surprise that those diseases that are caused from disorders of iron metabolism have abnormal iron studies; the iron studies reveal the diagnosis. If a definitive diagnosis is needed, a bone marrow biopsy can be performed. It should also come as no surprise that those diseases that are caused by disorders in globin synthesis, and therefore are agnostic to heme synthesis, have normal iron studies. Globin diseases are diagnosed by **hemoglobin electrophoresis** after iron studies are normal in the setting of microcytic anemia.

Iron Deficiency Anemia

Iron deficiency anemia is a problem where there is insufficient total body iron to keep up with the demands of erythropoiesis. While iron deficiency can theoretically be related to insufficient iron absorption (the person doesn't eat enough iron or has their duodenum resected), iron deficiency anemia generally is not caused by too little iron in. Instead, iron deficiency generally results from an increased demand for iron. Because the amount of iron absorbed is so small, and more or less fixed (yes, hepcidin does regulate iron absorption to some extent, but it can only regulate the amount of iron absorbed by about a factor of 4—from half the usual through twice the usual), if more than what is absorbed is needed by the marrow, the marrow will call the liver's stored iron to make up the difference. It drains ferritin. Increases in demand come from **pregnancy** and **slow, chronic, oozing bleeding**. We are not talking GI bleed, but rather microscopic amounts.

As a small number of red blood cells is lost over time, the body wants to maintain homeostasis at 14–16 g/dL. As hemoglobin falls a little, a little more erythropoietin is made. The production speed in the bone marrow increases a little. EPO inhibits hepcidin and a little more iron comes in from the diet. The iron from the diet isn't enough, however, so the bone marrow **uses ferritin** from the liver, starting to deplete the stored form of iron. An equilibrium is established—a small number of red blood cells is lost each day, but they are replaced with the increased turnover of the bone marrow. But eventually, since the diet cannot keep up with the production, **ferritin is depleted**. Recognizing the need for more iron, the liver increases the ability to collect iron, that is, it **increases circulating transferrin**. Transferrin goes from the liver (where there should be ferritin) to the bone marrow (which needs iron). But the hepatic stores are depleted. The transferrin goes to the enterocytes where there should be fresh iron, and there is, but not enough to fill all the seats on all that transferrin. Which means that most of the seats on transferrin are empty. Which means the **% sat is low**. And without any extra iron stores to feed the need of the marrow, **the serum iron is low**.

TIBC	High
Ferritin	Low
Iron	Low
% saturation	Low

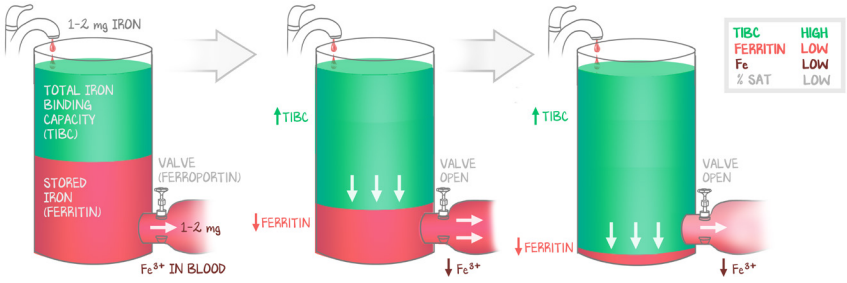

Figure 5.1: Iron Deficiency Anemia Mechanism
Using the iron silo, we can do the same thing again. The trickle input is fixed. The body needs more iron, so it opens the valve. More stored iron comes out out, the input fixed, so the silo drains until it is near empty, which means low ferritin. The silo is empty, ready to accept iron, which means high TIBC. There isn't any iron left in the stores, so the iron in the blood is low.

The way patients get chronic slow bleeds is either through **menometrorrhagia** (heavy or irregular menses) or by having **colon cancer**. Any male with iron deficiency anemia or any menopausal female with iron deficiency anemia has colon cancer until proven otherwise. Right-sided lesions tend to not cause symptoms but ooze as stool passes. The patient will not know until the hemoglobin gets quite low—the blood loss occurs slowly and allows the patient to adapt. Other patients who need more iron are those with chronic hemolytic anemia. Because of the chronic hemolysis, these patients depend on the increased production from the bone marrow to sustain equilibrium.

The complete blood count and the RBC indices **are reliable** for the diagnosis of iron deficiency anemia (and are not, on their own, reliable for any other diagnosis). A really **small MCV** (< 70) and a **huge RDW** is not pathognomonic, but it is extremely indicative of iron deficiency. Red blood cells that are small and have reduced pallor may be found on blood smear, but are nonspecific. Only the indices are that powerful. If you see a small MCV and a large RDW, choose iron deficiency anemia

Treatment is to locate the source of bleeding and stop it. Then **replete iron.** Usually repleting iron is by the oral route, but sometimes it could be intravenous, especially with patients with GI problems.

Anemia of Chronic Inflammatory Disease

Note that this is called anemia of chronic inflammatory disease, where other texts call it simply "chronic disease." You need the inflammation in the chronic disease to cause a microcytic anemia. Anemia of chronic kidney disease is normocytic. There is no such thing as anemia of bipolar, anemia of hypertension, or anemia of asthma. Those are chronic diseases but are not inflammatory diseases. The pathogenesis of an anemia caused by chronic inflammation relies on the inflammation, not just the diagnosis of any chronic disease.

Keeping within our framework created in Anemia #3: *Iron Regulation*, we know that hepcidin is regulated by inflammation via **interleukin-6**. As long as there is inflammation around, hepcidin is activated. Hepcidin turns off iron release into the blood. That means interleukin-6 restricts the amount of iron in the blood, thereby restricting the amount of iron that can be used by the erythroid progenitors. The **serum iron is low**. This chronic inflammation also turns off the ability for transferrin to circulate the stored form of iron. And because iron absorption cannot be turned completely off, iron is still brought by transferrin to the iron stores, where iron is stored as ferritin. Since the stored form of iron is increasing, that means there is an **elevated ferritin**. Fortunately, ferritin is also an acute inflammatory mediator, which can be used to remind you that ferritin is high in chronic inflammation. With the body's iron stores filling up, and hepcidin active, the liver does not feel the need to acquire more iron. And if the liver has enough, it thinks so does everyone else, so **transferrin decreases**. Existing transferrin becomes saturated by duodenal absorption, so the **% saturation increases**. However, all the while the erythrocyte precursors are starved for iron. It is all stored away as ferritin, so the RBC symptom is that of iron deficiency—small, hypochromic red blood cells.

TIBC	Low
Ferritin	High
Iron	Low
% saturation	Low

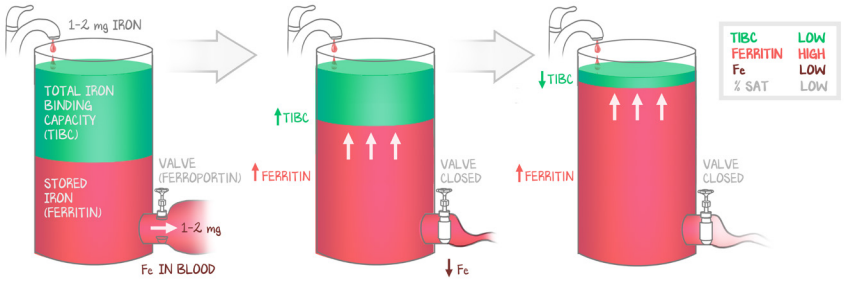

Figure 5.2: Anemia of Chronic Inflammatory Disease
Using the iron silo, we can do the same thing again. The trickle input is fixed. The body wants to hide the iron from bacteria, so it closes the valve. Less out, the same in, the silo fills, which means high ferritin. The silo is full, with very little room left, which means low TIBC. There is plenty of iron in the stores, but the iron cannot get out, so the iron in the blood is low.

The key distinguishing feature between iron deficiency and anemia of chronic inflammatory disease is the **ferritin**—elevated in inflammation, decreased in iron deficiency. The confirmatory diagnosis could be made by **bone marrow biopsy**, though this is rarely needed. In iron deficiency anemia there will be reduced iron in the marrow. In chronic inflammatory disease, there will be elevated iron (because some ferritin is in the bone marrow).

An elevated ferritin may be from any number of reasons. Ferritin elevates in chronic inflammatory diseases such as lupus, rheumatoid arthritis, or psoriasis. Ferritin elevates in chronic infection such as osteomyelitis. Ferritin can be elevated even in some malignancies. Treat the inflammation, treat the anemia.

One key distinction we need you to make is that chronic kidney disease does not cause anemia of chronic inflammatory disease. In chronic kidney disease, insufficient erythropoietin is synthesized and therefore hepcidin is not inhibited. The outcome (normocytic anemia) and the pathogenesis (low EPO, not high IL-6) are very different.

Thalassemias

Thalassemias are anemias caused by deficient production of globins. There are deficiencies of α-globin genes that cause α-thalassemias and point mutations of the β-globin genes that cause β-thalassemias. Thalassemia is the diagnosis—microcytic anemia with normal iron studies. All thalassemia is diagnosed with hemoglobin electrophoresis. All thalassemias present based on the number of genes missing or defective. Whether it is α-thal or β-thal is dependent on which chromosome is affected.

α-Thalassemia

α-Globin is coded for on two genes on each **chromosome 16**. α-Thalassemia results from **gene deletion** on chromosome 16. Since there are four copies of each gene in any one cell and there are four phenotypes of disease, we use 4 × 4 = 16 to separate it from β-thalassemia, which is on chromosome 11 (which is two ones next to each other, for two copies of β-globin genes). Because α-globin is used in all forms of hemoglobin, fetal and adult alike, it makes the most sense to have multiple copies of this gene. The outcome of this fact is simply a complicated table with α and – all over the place.

GENOTYPE	DISEASE STATE	PATIENT PRESENTATION
(α/α, α/α)	Normal	Normal
(α/α, α/−)	Asymptomatic carrier state	
(α/−, α/−) trans (α/α, −/−) cis	α-thal minor Mild anemia, no disease	Asymptomatic mild anemia
(α/−, −/−)	α-thal major, β_4 formation Transfusion dependent	Transfusion-dependent anemia
(−/−,−/−)	Hydrops fetalis Incompatible with life	Death

Table 5.1: α-Thalassemia

The good news is that, really, there are only two disease states. **Hydrops fetalis is fatal in utero**, so you never see it (like β_4 discussed below, in hydrops fetalis, γ_4 (named hemoglobin Barts) forms, which is fatal to erythrocytes). **Asymptomatic carrier state** is completely asymptomatic, so you never see this state, either. Even if you did "see it" with your eyes, if you ordered a hemoglobin, it would be normal; if you ordered a hemoglobin electrophoresis, it would be normal, too.

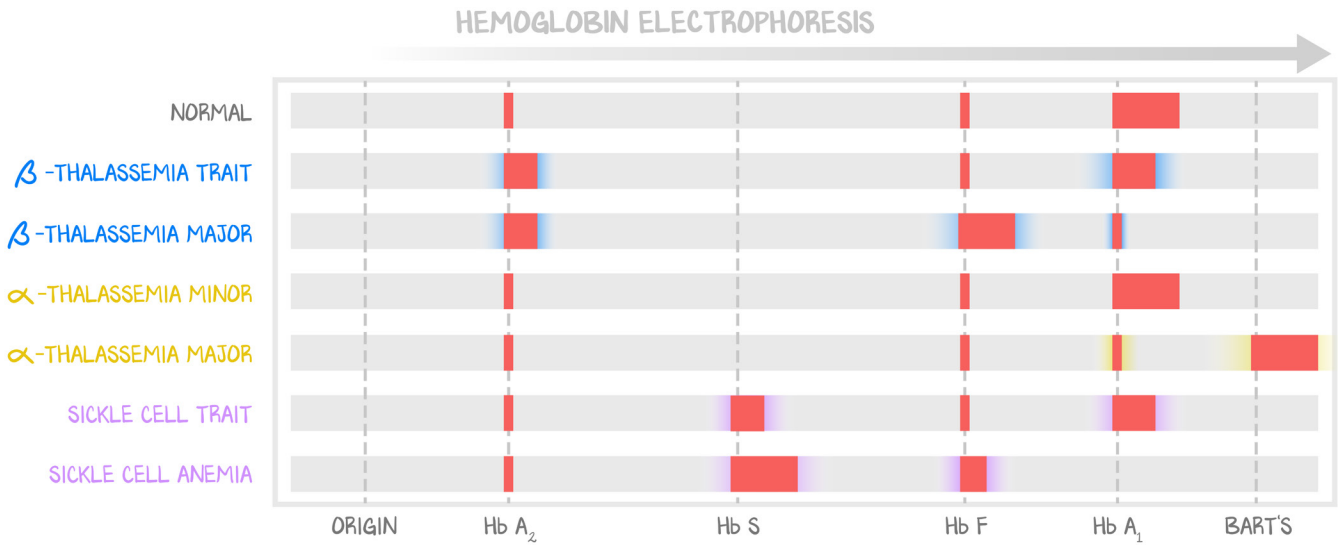

Figure 5.3: Hemoglobin Electrophoresis
This illustration demonstrates conceptually how hemoglobin electrophoresis facilitates the diagnosis. From left to right, proteins precipitate out allowing their location on the bar to indicate which hemoglobin is there. The width of the bar denotes quantity. Normally, there is a lot of HbA1 as indicated by the thick red line, and nominal amounts of HbA1 and HbF, indicated by the small red lines in those positions. In β-thalassemia minor, there is less HbA1 and more HbA2. In β-thalassemia major, there is no HbA1 and massively increased HbF. In α-thalassemia minor, there is a normal electrophoresis. In α-thal major, with three genes deleted, there is hardly any HbA1, HbA2, or HbF, but there is the presence of hemoglobin Barts, not found in any other disease. In sickle cell trait there is some HbS, but plenty of HgbA1. In sickle cell disease, there is a lot of HbS, no HbA1, and an increased HbF.

That means you will see only two types of patients. **α-thal minor** presents with a mild anemia, hemoglobin never below 10 g/dL, which in turn means never-should-be-evaluated. They don't need transfusions, and they don't need medical management. The inheritance patterns do have associations with geographic location of origin. The **trans type**, where one α-globin gene is deleted on each chromosome, is prevalent in Africa. The **cis type**, where two α-globin genes are deleted from the same chromosome, leaving the other chromosome normal, is more common in Asia.

α-Thal major is **transfusion dependent**. The significantly disproportionate amount of β-globin compared to α-globin induces the formation of β_4, known as hemoglobin H, abbreviated HbH. HbH has an extremely high affinity for oxygen, and therefore is not useful for oxygen delivery—any oxygen it picks up in the lungs it refuses to release in the tissues. Additionally, HbH is prone to oxidation, which causes it to precipitate and form intracellular inclusions that promote splenic destruction. First, there is reduced hemoglobin in general. Second, the hemoglobin that is made causes the RBCs that have it to be lysed. This combination of decreased production AND increased destruction means the hemoglobin will be low. All the time. That requires transfusions. With the need for regular (usually monthly) transfusions, **iron overload is an inevitability**. With **iron chelators** life can be extended into the second or third decade of life. Genetic counseling is a must.

β-Thalassemia

β-Globin is coded for on a gene located on **chromosome 11**. Since every cell has two copies of each chromosome, there are two β-globin genes. Mutations result in either decreased (but detectable) β-globin production or no globin production from that gene. Mutations on chromosome 11 come in the way of **point mutations** at **splice sites** (usually introns) and in **promoter regions**. Those mutations that occur at splice sites generate mRNA that produces unusable protein, and are generally the mutations that prohibit β-globin synthesis from that gene. Those that mutate the promoter regions merely reduce the amount produced but do not eliminate it.

β^+-Thalassemia results in an impaired but detectable amount of β-globin synthesis derived from its gene. β^0-Thalassemia results in an undetectable amount of β-globin synthesis derived from this gene. While this allows for multiple permutations (wild type, β^+, and β^0), clinically, this distinction between β^+ and β^0 is not appreciated. A heterozygote (β^+/β or β^0/β), regardless of the type of mutation, presents with a mild disease, called **β-thalassemia minor**, or **β-thalassemia trait**. Patients are asymptomatic, do not require transfusions, and do not have the syndrome of ineffective hematopoiesis. Homozygotes (β^+/β^+ or β^+/β^0 or β^0/β^0), regardless of the severity of the mutation, present with a severe disease, **β-thalassemia major**. Since β-thalassemia minor is asymptomatic, it need not be discussed further.

Impaired β-globin synthesis results in anemias by two distinct, simultaneous mechanisms—extravascular hemolysis and ineffective erythropoiesis.

Extravascular hemolysis means that there is a component of hemolytic disease. We have excluded it from the discussion on hemolytic anemias because we want you learning that hemolysis results in normocytic anemia. However, in β-thal major, without any β-globin made, α-globins have nothing to pair with, so they form α_4, insoluble α-globin aggregates. These α-globin aggregate-filled RBCs are marked for destruction by the spleen. This contributes to the already massively compromised production anemia.

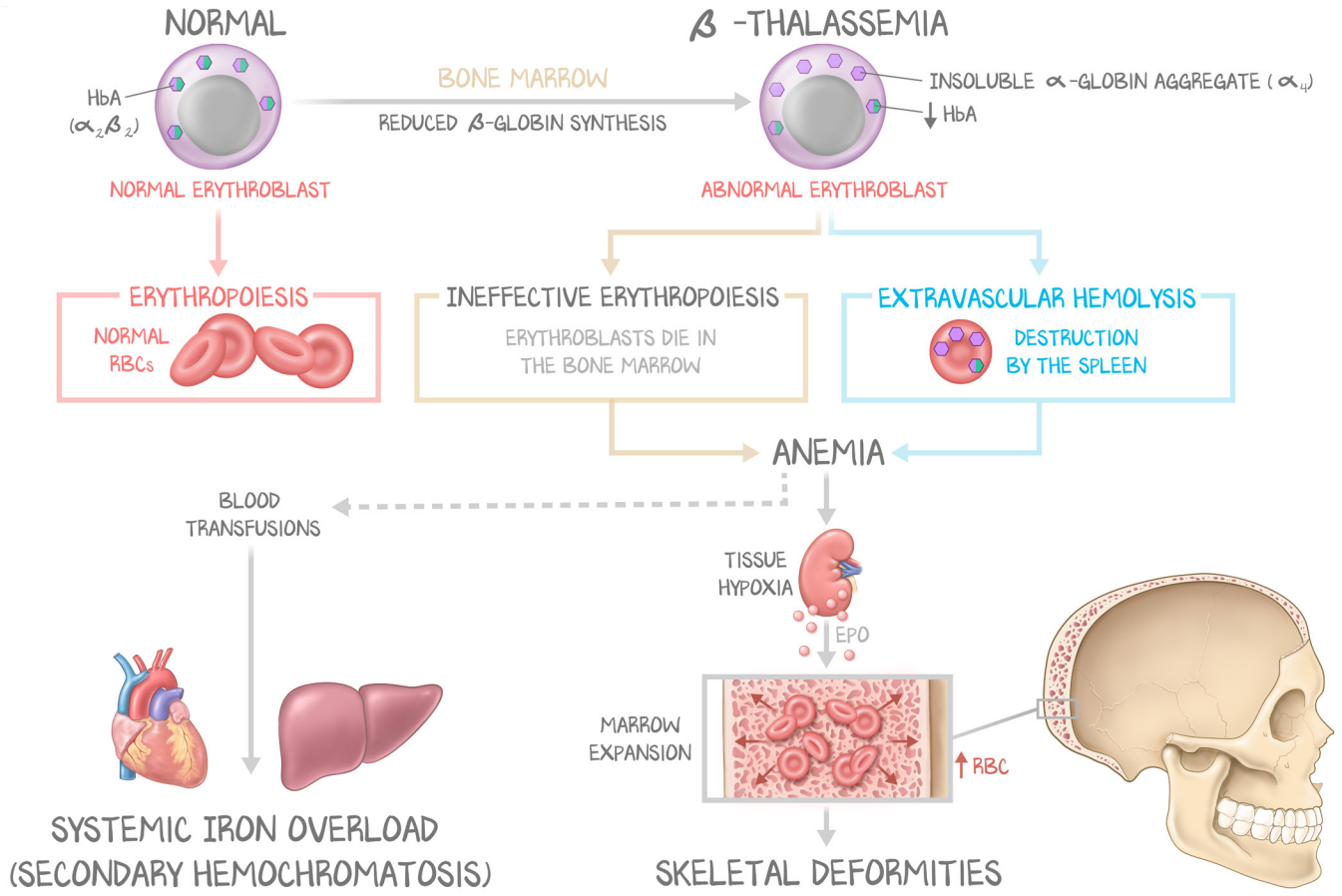

Figure 5.4: Extramedullary Hematopoiesis Complications
Normally HbA1 allows for normal erythrogenesis. In β-thalassemia major, reduced β-globin synthesis causes a deficiency of HbA1, but more importantly allows for the accumulation of α-globin tetramers. These are identified as a bad erythroblast in the marrow, leading to ineffective erythropoiesis—not enough cells get out of the marrow and into the blood. Those that get to the blood are identified as bad erythrocytes in the blood, so are hemolyzed by macrophages in the reticuloendothelial system (the spleen, liver, bone marrow). Both contributes to catastrophic anemia requiring blood transfusions regularly (leading to hemosiderosis, iron overload) and an upregulated EPO signal (leading to marrow expansion and skeletal deformities).

Ineffective erythropoiesis drives most of the disease's syndromes. First, the α-globin aggregates impair erythrocyte development, so very few erythrocytes even leave the marrow. Those that do leave have a shortened life span because of extravascular hemolysis. Without β-globin synthesized, **hemoglobin cannot be synthesized**, leaving the red blood cells that are dispatched from the marrow severely lacking in hemoglobin. Thus, they are hypochromic and show central pallor (something that you have seen in every microcytic anemia disease). The woeful loss of circulating hemoglobin results in increased expression of EPO. EPO stimulates potentially hematopoietic sites into hematopoiesis, resulting in **marrow expansion** (skeletal deformities) and **extramedullary hematopoiesis** (hepatosplenomegaly). Skeletal deformities are in the way of non-long bones elongating to accommodate marrow formation.

Patients die without blood transfusions. The natural course of the disease is to encounter a **hemoglobin of 3–6 g/dL** starting around **age 6–9 months**, when the marrow switches from HbF ($\alpha_2\gamma_2$) to adult hemoglobin A ($\alpha_2\beta_2$). With regular (monthly) transfusions, each transfusion containing enough iron for 6 months, **iron overload is an inevitability**. With iron chelation therapy, life into the third or fourth decade is possible. Genetic counseling is a must. Because the blood smear is hypochromic and microcytic, this diagnosis would be missed in the time before computers, when diagnosis was based on a blood smear and your eyes. Because we arrive at the diagnosis of microcytic anemia using the hemoglobin, MCV, and

reticulocyte count from the CBC, then reveal that the iron labs are normal (because this is a deficiency of globin, not heme), we are no longer fooled. The only diagnosis that has that laboratory approach is thalassemia. Thalassemias are diagnosed by **hemoglobin electrophoresis**. Electrophoresis of β-thal major would show no HbA, abundant HbF, and some, usually normal, amount of HbA2.

DISEASE	NOTES
Iron deficiency anemia	Low ferritin, High TIBC, Low Iron Hepcidin will be low (not used for diagnosis) Dietary deficiency unusual Increased demands from pregnancy and hemolytic anemia Give iron
Anemia chronic inflammatory disease	High ferritin, Low TIBC, Low Iron Hepcidin will be high (not used for diagnosis) Chronic disease must be inflammatory
α-Thal	Normal iron labs, normal hepcidin (not used for diagnosis) Each chromosome 16 has 2 copies each, total of 4 genes, deletion Electrophoresis either normal or shows HbH 1 gene deleted, asymptomatic 2 gene deleted, thal minor, anemia > 10 3 gene deleted, thal major, skel deformities, transfusion dependent 4 gene deleted, hydrops fetalis, fatal in utero
β-Thal	Normal iron labs, normal hepcidin (not used for diagnosis) Each chromosome 11 has 1 copy each, total of 2 genes, promoter Electrophoresis shows reduced A2, increased F 1 gene deleted, thal minor, anemia > 10 2 gene deleted, thal major, skel deformities, transfusion dependent

Table 5.2